E-17-633
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1/5/2006

FINAL REPORT on Whitaker Foundation sponsored research project (TF-03-0021)

Quantitative Analysis of Targeted Retrovirus Infection (PI: Joseph M. Le Doux, PhD)

I. ABSTRACT

Why the project was undertaken

Recombinant retroviruses are one of the most commonly used means to transfer genes in gene therapy clinical trials because they can permanently integrate a therapeutic gene into the chromosomal DNA of target cells, resulting, in principle, in a long-term cure. Unfortunately, the current generation of recombinant retroviruses often are not able to both efficiently and selectively transfer genes to cells. Given the large number of important clinical applications, there is significant motivation to improve the efficiency and selectivity of retroviral-mediated gene transfer. Successful gene transfer with retroviruses requires the completion of a complex series of steps that begins with binding of the virus to the cell, transport of the bound virus to a location where its cellular receptors are expressed, followed by an interaction between the envelope proteins of the virus and their cellular receptors that leads to fusion of the virus with the cell and its entry into the cytoplasm. The mechanisms by which these steps of infection occur are poorly understood. It is important that we understand the mechanism by which retroviruses accomplish these early steps of infection because such knowledge could lead to the development of more efficient and selective strategies for genetically modifying cells in human gene therapy protocols, or for blocking infection by wild-type, pathogenic retroviruses. Therefore we undertook the Whitaker project in order to make progress towards our *long-term goal*, which is to establish engineering-based strategies for improving the functionality of recombinant retroviruses and lentiviruses for the purposes of human gene therapy.

What research was performed

We had two major findings. First, we found that the route that retroviruses take within a cell is controlled by the receptors and co-receptors that they interact with when they bind to the cells they are infecting. In addition, the probability that they will successfully infect a cell is a function of the intracellular pathway they take. We showed this be developing a novel experimental system that was composed of several cell lines that expressed retrovirus receptors that were identical in the portion that interacts with viruses, but different in the portion that controlled how the receptors were trafficked within the cell. We engineered retroviruses to bind to these receptors, and studied the effect of receptor trafficking on virus trafficking and infection. Our second major finding was that retroviruses activate intracellular signals when they bind to cells. Specifically, they activate rac1, a molecule that coordinates the formation of actin filaments within the cells. We showed that viruses induce this signaling via proteoglycans on the surface of the virus and via integrins on the surfaces of the cells. We are currently investigating the role that rac1 activation has in retrovirus transduction.

Why the project was important

The project was important because it showed that the molecules that are on the surface of retroviruses dictate how the viruses will traffick within the cell, and whether or not they induce intracellular signaling. Our findings suggest that by manipulating the surface properties of

retroviruses, we should be able to control the signals they induce within cells, and how the viruses are transported within the cells, and thereby control the outcome of gene transfer, either making it more efficient, more selective, or both. The ability to do this will enable us to develop safer, more effective human gene therapy protocols.

II. PAPERS and POSTERS from project

A. Papers

Krishna, D., and Le Doux, JM, "Murine Leukemia Virus Particles Activate Rac1 in HeLa Cells", in preparation

Krishna, D., Raykin, J, and Le Doux, JM, "Targeted Receptor Trafficking Affects the Efficiency of Retrovirus Transduction", Biotechnology Progress, 21(1): 263-273 (2005)

B. Posters

Krishna, D., Le Doux, J.M. (2002). An Experimental System for the Analysis of Targeted Retrovirus Transduction. *The Second Joint Meeting of the IEEE Engineering in Medicine and Biology Society and the Biomedical Engineering Society, Houston, Texas.*

Krishna, D., Le Doux, J.M. (2003). An Experimental System for the Analysis of Targeted Retrovirus Transduction. *The 7th Annual Hilton Head Workshop, ET2003: Engineering Tissues, Hilton Head, S.C.*

Krishna, D., Coburn L., Sheng J., Rubin D.H., Hodge T.W., Le Doux, J. M. (2003) The development of an experimental system to investigate the role of host cell factors in retrovirus transduction. *The 6th Annual American Society for Gene Therapy Meeting, Washington D.C.*

Krishna, D., Coburn L., Sheng J., Rubin D.H., Le Doux, J. M. (2003) An experimental system to investigate the role of host cell factors in retrovirus transduction. *The Biomedical Engineering Society Annual Meeting, Nashville, TN*

Krishna, D, Le Doux, J.M. (2004). Receptor trafficking affects the efficiency of retrovirus transduction. *The 7th Annual American Society for Gene Therapy Meeting.*

Krishna, D, Le Doux, J.M. (2004). Receptor trafficking affects the efficiency of retrovirus transduction. *2004 Annual Meeting, American Institute of Chemical Engineers.*

Krishna, D, Le Doux, J.M. (2005). Targeted receptor trafficking affects the efficiency of retrovirus transduction. *The 8th Annual American Society for Gene Therapy Meeting.*

Krishna, D, Le Doux, J.M. (2005). Retrovirus particles activate cell signaling protein Rac1 upon binding to target cells. *Georgia Life Sciences Summit.*

III. GRANTS

Continuing support for Whitaker project
None, but I am currently preparing an R01 that is based on the Whitaker project data.

New projects funded by major grants
CAREER: Engineering Recombinant Lentiviruses for Cystic Fibrosis Gene Therapy
National Science Foundation, 2002-2007

Induction of Stem Cells to Adopt an Endocrine Fate
National Institutes of Health (R21), 2002-2004

Rapid Virus Concentration and Purification for Enhanced Throughput and Sensitivity of Molecular and Diagnostic Virus Assays
Coulter Foundation, 2005-2006